Addition of Glucose Oxidase for the Improvement of Refrigerated Dough Quality

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ABSTRACT

Cereal Chem. 91(6):548–553

Refrigerated dough encompasses a wide range of products and is a popular choice for consumers. Two of the largest problems that occur during refrigerated dough storage are dough syruping and loss of dough strength. The goal of this study was to evaluate glucose oxidase as an additive to refrigerated dough with the purpose of maintaining dough strength and retarding dough syruping. Refrigerated dough was evaluated for the degree of dough syruping (DDS), dough strength, rheological characteristics, baking quality, and protein quality. The addition of glucose oxidase at 10 mg/kg was able to significantly ($P < 0.05$) reduce dough syruping and maintain the strength of the dough. Addition of glucose oxidase at 5 and 25 mg/kg was not able to reduce the level of dough syruping at a satisfactory level. Degradation of protein was found to occur during storage of refrigerated dough. DDS had a negative correlation ($r = –0.60$ to $–0.94$) to the level of polymeric proteins and a positive correlation ($r = 0.60$ to 0.98) to the low-molecular-weight proteins. Overall, glucose oxidase at 10 mg/kg can improve refrigerated dough quality by reducing dough syruping and maintaining dough strength.

Refrigerated dough encompasses a wide range of products including bread, rolls, pastries, and pizza crust and is a popular choice for consumers. Two of the largest problems that occur during refrigerated dough storage are dough syruping and loss of dough strength. Dough syruping occurs when degradation of dough components, mainly arabinoxylan and proteins, results in liquid being released from the dough during storage (Courtin et al 2006; Simsek et al 2010). Previously, prevention of dough syruping was attempted by removing the bran before milling (Gys et al 2004), by choosing flour with low xylanase activity, by choosing wheat variety or growing environment (Simsek et al 2011a, 2011b), and by adding xanthan gum to sequester water (Simsek 2009). Although these methods may result in the reduction of dough syruping, they may not be able to address the loss of dough strength during storage of refrigerated dough.

Addition of glucose oxidase (GOX) counteracts excessive xylanase actions that could substantially reduce dough consistency and increase stickiness (Martínez-Anaya and Jiménez 1998). Xylanases interfere with the cross-linking of high-molecular-weight arabinoxylans and increase the release of entrapped water by cleaving arabinoxylan complexes, and GOX corrects for this effect by enhancing the cross-linking of proteins and arabinoxylans (Primo-Martin et al 2005). GOX is an oxidizing agent that produces hydrogen peroxide as an intermediate reaction product. The hydrogen peroxide produced by GOX reacts with free thiols in cysteine, peptides, and proteins and with phenolic compounds in arabinoxylan. GOX has been traditionally used as an oxidant in bread-baking systems to increase dough strength and elasticity (Hanft and Koehler 2006). Several studies have been conducted by various researchers to determine the effects and reaction mechanisms of GOX in wheat flour and dough systems. It has been theorized that GOX acts as a catalyst in the oxidation of α-D-glucose to α-D-gluconolactone and hydrogen peroxide. The hydrogen peroxide then reacts with the thiol groups of the gluten proteins, creating disulfide bonds and linking ferulic acid residues that are attached to arabinoxylan (Hanft and Koehler 2006; Steffolani et al 2010).

Previous studies have shown that GOX has significant effects on the protein in wheat flour dough. Steffolani et al (2010) found that the addition of GOX resulted in a significant ($P < 0.05$) decrease in the SDS-soluble proteins, which in turn led to a rise in the SDS-insoluble protein aggregates. This changing protein composition was a result of the formation of disulfide and nondisulfide cross-linking of the proteins, which was also shown by Hanft and Koehler (2006). The effect of GOX on cross-linking and proteins is represented by increased association of pentosans into the insoluble glutenin protein matrix such as glutenin macropolymer (Primo-Martin et al 2005; Steffolani et al 2010) and modifying the electrophoretic pattern of protein fractions. Bonet et al (2007) reported that GOX improved breadmaking functionality of dough that was damaged by protease from insect infestation.

The literature indicates that GOX is a promising candidate to ameliorate or prevent deterioration of dough quality during preservation, such as syruping and viscosity changes, because of its counteraction against xylanase, enhancement of water sequestration, and polymerization of proteins through oxidative gelation of proteins and arabinoxylans. Despite this potential, there has been limited research on the effect of GOX addition on refrigerated dough quality. The goal of this study was to evaluate GOX as an additive to refrigerated dough with the purpose of maintaining dough strength and retarding dough syruping.

MATERIALS AND METHODS

Materials. Commercial hard red spring wheat flour with no oxidizing agents or other additives was obtained from North Dakota State Mill (Grand Forks, ND, U.S.A.). Gluzyme mono 10000 BG GOX (Novozymes, Bagsvaerd, Denmark) was supplied by Lallemand Baking Solutions (Montreal, QC, Canada). GOX activity was 100,000 U/g, and 1 U of GOX is defined as the amount of enzyme that oxidizes 1 μmol of *o*-dianisidine per minute at 25°C. All other chemicals were at least ACS grade and from Sigma-Aldrich (St. Louis, MO, U.S.A.).

Dough Preparation. The dough was prepared by using commercial hard wheat flour from North Dakota State Mill with no additional oxidation or additives according to the method of Simsek et al (2011a). GOX was added as a liquid suspension (1 mL) at 5, 10, and 25 mg/kg levels. The flour was mixed with GOX, sodium chloride (1.8%), and water with 0.2% sodium azide (according to the farinograph absorption at 500 BU). Refrigerated dough samples without GOX were also prepared and tested as a control. The dough was stored in centrifuge tubes or plastic zip-top bags at

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4°C for up to 34 days. Sample analysis was done on days 0, 1, 2, 3, 6, 10, 16, and 34.

Dough Syruping. Pieces of dough (10 g) were stored in centrifuge tubes at 4°C for 34 days. Samples were taken from the refrigerator and centrifuged at 22,000 × g for 30 min. Prior to centrifuging, the weight of each tube plus sample was measured. After centrifuging, the supernatant was carefully removed with a pipette, and tissue was used to wipe the sides of the tubes. The degree of dough syruping (DDS) was determined by the difference in weight after removal of the supernatant (Gys et al 2003).

Dough Strength. Dough strength was measured by determining the resistance to extension using a texture analyzer with a Kieffer microextension rig according to the method of Kieffer et al (1998). Prior to analysis, the dough samples were removed from the refrigerator. Then the dough pieces (10 g) were placed into the mold and rested for 40 min. The mold pressed the dough into several strips, which were approximately 4 mm in width by 50 mm in length. Dough strips were placed into the microextension rig and stretched vertically. The resistance to extension was measured as force against the hook in grams.

Dough Rheology. The elastic (G') and viscous (G'') moduli were measured with a rotational rheometer (Stresstech, ATS Rheosystems, Bordentown, NJ, U.S.A.) by dynamic oscillatory rheometry according to the method of Zhang et al (2011). Pieces of dough were cut (0.2 g) from the larger dough ball and placed between the parallel plates. The gap was lowered to a height of 1.2 mm, and the excess sample was cut off from the outside of the plates. Then the plate was lowered to a final gap height of 1.0 mm.

Baking. Pup loaves (25 g) were prepared according to U.S. patent 6,149,960 (Book et al 2000) with a few modifications. The formula was as follows: flour, 25 g; water, 16 mL; salt, 0.45 g; shortening, 0.75 g; sodium bicarbonate, 1.18 g; glucono-δ-lactone, 0.5 g; and Levn-Lite sodium aluminum phosphate, 1.08 g. After mixing, samples were molded into loaves, placed in pup loaf pans, and stored in vacuum-sealed bags over a period of 34 days. The refrigerated dough bread loaves were removed from the refrigerator and rested for 10 min and then baked at 225°C for 20 min. The loaf volume was measured after baking with rapeseed displacement (AACC International Approved Method 10-05.01).

High-Performance Size-Exclusion Chromatography (HP-SEC). Dough samples for protein characterization were frozen for 24–48 h and then lyophilized and ground before analysis. Extractable protein was obtained following the method of Gupta et al (1993) with minor modifications (Ohm et al 2006). Flour (10 mg, 14% mb) was suspended in 1 mL of extraction buffer (0.5% SDS and 0.1M sodium phosphate, pH 6.9) and stirred (5 min, 2,000 rpm) with a pulsing vortex mixer to solubilize extractable protein. Then the mixture was centrifuged (15 min at 17,000 × g) and filtered through a 0.45 μm polyvinylidene fluoride membrane. The sample was immediately heated for 2 min at 80°C to inactivate endoprotease (Larroque et al 2000). The unextractable protein was solubilized from the residue after extracting the extractable protein with a sonicator (30 s in 1 mL of extraction buffer solution at the power setting of 10 W output). The solubilized unextractable protein was prepared with the same conditions as the extractable protein. HP-SEC was performed with an Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA, U.S.A.). The extractable and unextractable protein were separated by a narrow-bore size-exclusion column (300 × 4.5 mm, BIOSEP SEC S4000, Phenomenex, Torrance, CA, U.S.A.) with guard cartridges (BIOSEP SEC S4000) (Batey et al 1991; Ohm et al 2009). Proteins were eluted by 50% acetonitrile in water with 0.1% trifluoroacetic acid at a flow rate of 0.5 mL/min and detected at 214 nm with a photodiode array detector (1200, Agilent Technologies). These experiments were duplicated, and the mean values were used for data analyses.

Absorbance data from HP-SEC of protein extracts were analyzed with MATLAB statistical software (MATLAB 2013, Math-

Works, Natick, MA, U.S.A.) (Ohm et al 2006). Absorbance values were interpolated to 0.002 min intervals by a cubic spline procedure in MATLAB software, which is a method of calculating new data points within the range of previously determined data points by using a B-spline function. Absorbance area was calculated by mean absorbance by time interval of 0.002 min with the interpolated absorbance values. Data collection was performed by using the sum of absorbance area for each retention time interval of 0.01 min between 3.6 and 9 min of run time. The absorbance area values for total proteins were mathematically estimated by adding absorbance area values of extractable protein (Ohm et al 2009). Absorbance area percentage values were calculated for each retention interval of 0.01 min over the total absorbance area (Ohm et al 2006). Simple linear correlation coefficients (r) were calculated between wheat parameters and absorbance area percentage values and are presented as a continuous spectrum over retention time.

Statistical Analysis. Statistical analysis was performed with the SAS System for Windows (version 9.2, SAS Institute, Cary, NC, U.S.A.). SAS software was used to determine analysis of variance with a completely random design, and mean separation was done using least significant difference (α = 0.05).

RESULTS AND DISCUSSION

DDS. Dough syruping is one of the most important quality factors when evaluating refrigerated dough. The DDS for refrigerated dough prepared with GOX is shown in Figure 1. For the dough with no GOX the DDS ranged from 0.69 to 20.96% from day 0 to day 34 of refrigerated storage. This level of dough syruping falls within the ranges that have previously been found in other studies (Gys et al 2003; Simsek et al 2011a, 2011b). The DDS on day 0 was less than 1% for all treatments. However, the dough prepared with 10 mg/kg of GOX had 0.35% DDS, which was about half that of the dough with no GOX (0.69% DDS). For all samples, the level of DDS reached a plateau at day 16 of refrigerated storage. This trend has previously been seen in other refrigerated dough research studies (Gys et al 2003, 2004; Simsek 2009; Simsek et al 2011b). After a certain maximum of water is released and maximum degradation of the dough quality occurs, the DDS begins to level off. The refrigerated dough can only release as much water as was put into the system in the beginning. The amount of water retained by the refrigerated dough during storage and the rate at which the dough releases the water are dependent on many complicated factors. These factors include wheat variety, growing environment (Simsek et al 2011a, 2011b), arabinoxylan population (Gys et al 2003; Courtin et al 2006;
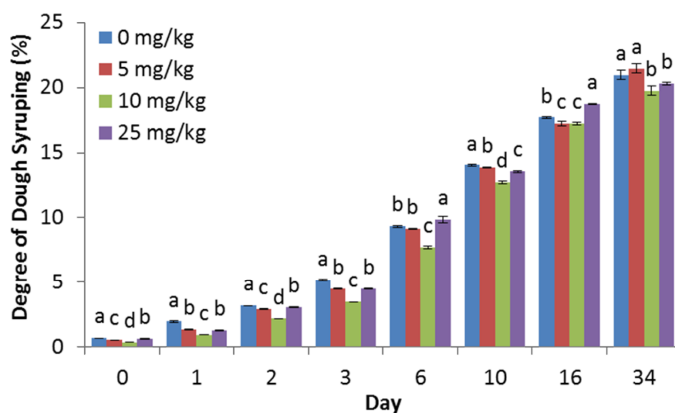

Fig. 1. Degree of dough syruping (%) for refrigerated dough with different levels of glucose oxidase. Treatments on the same day with the same letter are not significantly different ($P > 0.05$). Error bars represent standard deviation.

Simsek et al 2010), and xylanases (Gys et al 2004; Courtin et al 2006).

During the period of refrigerated storage, the 10 mg/kg of GOX treatment maintained significantly ($P < 0.05$) lower DDS than the dough with no GOX. The DDS of the dough treated with 10 mg/kg of GOX ranged from 0.35 to 19.75% from day 0 to day 34 of refrigerated storage. Treatment with 5 mg/kg of GOX resulted in significantly lower ($P < 0.05$) DDS than the dough with no GOX from day 0 to day 3, but at day 6 the DDS of the dough with 5 mg/kg of GOX increased so that it was no longer significantly ($P < 0.05$) lower than the untreated dough. The dough treated with 25 mg/kg of GOX had a similar trend as the 5 mg/kg treatment. Addition of GOX must be done carefully to avoid over- or undertreatment, which results in less than optimal control of dough syruping. Also, supplementation of refrigerated dough with GOX may be best used with flours that have lower initial DDS levels or in addition to other treatments to control dough syruping.

Dough Strength and Rheological Characteristics. Changes in dough strength during storage were determined by measuring
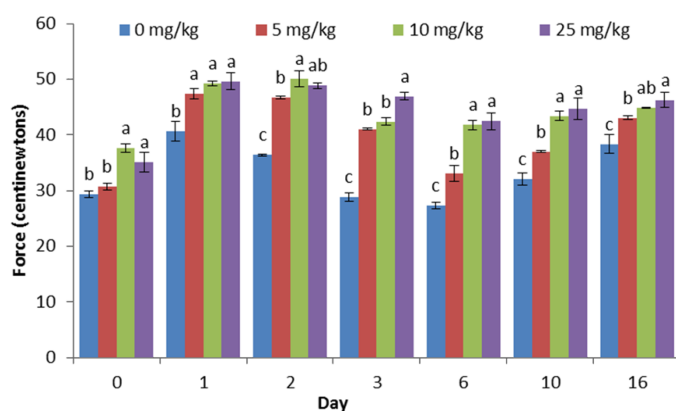

Fig. 2. Resistance to extension in refrigerated dough with different levels of glucose oxidase. Treatments on the same day with the same letter are not significantly different ($P > 0.05$). Error bars represent standard deviation.

the resistance to extension by using a texture analyzer with a Kieffer microextension rig (Kieffer et al 1998). For day 1 through day 16, treatment with GOX (at all levels) resulted in significantly ($P < 0.05$) higher resistance to extension (Fig. 2). The control refrigerated dough (0 mg/kg of GOX) showed much larger fluctuations in dough strength during the storage period than the doughs treated with GOX. The addition of GOX seemed to result in the preservation of dough strength during refrigeration. The changes in dough strength were likely because of moisture migration in the dough system.

Dough strength during storage of the refrigerated dough showed a similar trend to the results found by Zhang et al (2011). The resistance to extension increased slightly for the first two days of storage and then began to decrease. There was no significant difference ($P > 0.05$) in resistance to extension for 10 and 25 mg/kg of GOX treatments, except for on day 3. However, the higher DDS observed for the 25 mg/kg of GOX treatment makes it unsuitable for refrigerated dough production. These results showed that the most important benefit of GOX to refrigerated dough quality was the retention of dough strength. Loss of dough strength will result in a baked product with lower volume and a denser crumb (Bonet et al 2006; Hanft and Koehler 2006). However, this is another case in which addition of GOX at excessive levels will result in tougher dough. The tougher dough will have lower extensibility, hinder gas cell expansion, and cause lower end-product volume (Hanft and Koehler 2006).

Dough strength during storage was also determined by measuring the rheological properties of the dough with a rheometer.

The rheological properties of the dough were also evaluated to assess changes in dough quality during storage. Figure 3 shows the elastic modulus (G'), and the viscous modulus (G'') is presented in Figure 4. The G' was higher than the G'' for all levels of GOX and during all storage points. This comparison revealed that the dough had a more elastic than viscous nature, which was typical for dough produced from hard spring wheat flour (Payne 1987).

There were some differences in rheological behavior of dough containing different levels of GOX. In all treatments, G' and G'' decreased during storage of the dough. Zhang et al (2011) found similar changes to the elastic and viscous moduli in refrigerated
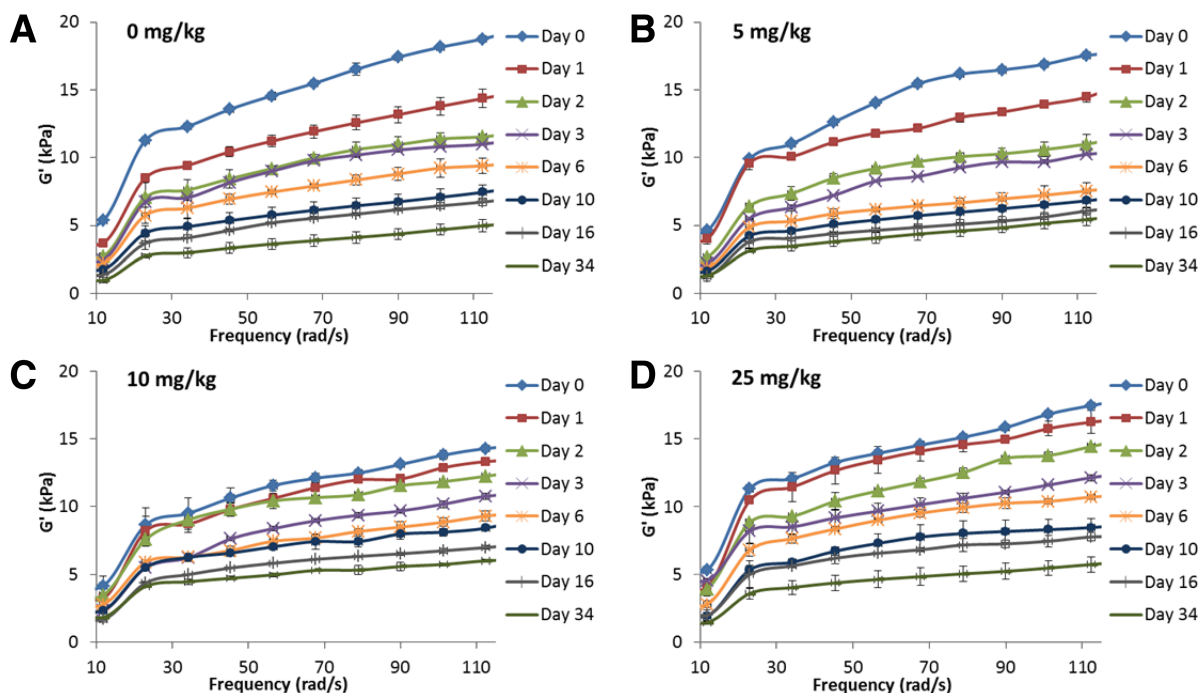

Fig. 3. Elastic modulus (G') of refrigerated dough with glucose oxidase at different levels: **A,** 0 mg/kg; **B,** 5 mg/kg; **C,** 10 mg/kg; and **D,** 25 mg/kg. Error bars represent standard deviation.

dough during storage. The elastic modulus (G') decreased because of degradation of the gluten proteins in the dough, resulting in lower dough strength, which was also evident from the microextensibility results (Fig. 2) for refrigerated dough prepared without GOX. However, the results of fundamental rheological analysis and the microextensibility test will show slightly different patterns. This difference is because the rheometer measures small strain deformation, the microextension rig measures large strain deformation, and the geometries of the tests are different, so the forces act in different ways on the dough (Dunnewind et al 2003; Tronsmo et al 2003). In the case of the decrease in the viscous modulus, the water loss from dough syruping results in a dryer dough, which will have lower viscosity. Treatment with 10 mg/kg of GOX resulted in much less change to the rheological properties of the dough during storage (Figs. 3C and 4C). The oxidative action of the hydroperoxide produced by the GOX and the lower DDS resulted in the preservation of the rheological characteristics of the refrigerated dough with 10 mg/kg of GOX. The G' and G'' of refrigerated dough with 0, 5, and 25 mg/kg of GOX were similar to each other and showed more dramatic change during storage than the 10 mg/kg of GOX treatment. Although treatment with higher levels of GOX should also result in preservation of dough rheological quality, the increased DDS in the sample with 25 mg/kg of GOX would cause the changes in the G' and G'' seen in this study. Wikström and Eliasson (1998) reported significant changes to rheological properties of wheat flour dough with GOX treatment. However, it was determined that the effect of GOX is dependent on the flour characteristics, and because GOX

uses glucose as a substrate, the amount of glucose in the dough will influence the effectiveness of this enzyme (Wikström and Eliasson 1998). For this reason, it may be beneficial to determine the free glucose content of flour and add glucose to the formulation when using GOX in refrigerated dough. However, in this study the focus was on determining the effects of GOX on refrigerated dough without any confounding factors. Also, the addition of glucose to the refrigerated dough formula as a substrate for GOX would require further investigation and adjustment to determine the optimum level of GOX to add to the formula.

End-Product Quality. Pup loaves (25 g of flour) were prepared and stored in vacuum-sealed packages to determine the stability of the end product prepared from refrigerated dough treated with GOX. These results are shown in Table I. In general, GOX resulted in improved stability of the end-product dough and increased volume of the baked product. On day 0 the volumes of the pup loaves prepared from refrigerated dough containing 5, 10, and 25 mg/kg of GOX were all significantly ($P < 0.05$) higher than the loaf with no GOX (Table I). The loaf volumes of the samples with no GOX ranged from 125 to 94 cm³ during the 34 day storage period. The loaves with 5 and 10 mg/kg of GOX had similar loaf volumes ranging from 131 to 103 and from 134 to 109 cm³, respectively. The treatment with 25 mg/kg of GOX resulted in decreased loaf volume (132–101 cm³) during storage of the refrigerated dough. Although the addition of 25 mg/kg of GOX may have improved the strength of the gluten proteins in the refrigerated dough, the increased DDS in these samples likely caused the decrease in loaf volume. Treatment with 10 mg/kg of
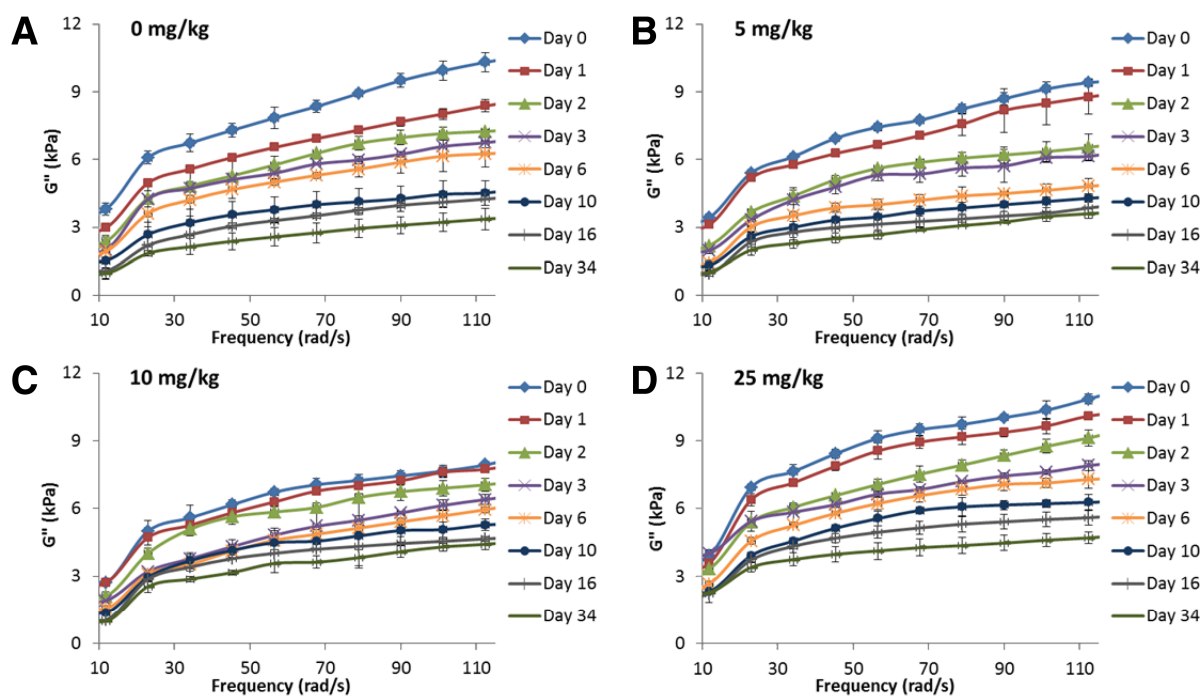

Fig. 4. Viscous modulus (G'') of refrigerated dough with glucose oxidase at different levels: **A,** 0 mg/kg; **B,** 5 mg/kg; **C,** 10 mg/kg; and **D,** 25 mg/kg. Error bars represent standard deviation.

TABLE I
Loaf Volume (cm³) of 25 g Pup Loaves Baked from Refrigerated Dough with Glucose Oxidase (GOX)[y]

GOX Level	Day 0	Day 1	Day 2	Day 3	Day 10	Day 16	Day 34	% Decrease in Volume[z]
0 mg/kg	125b	133ab	129b	126ab	110b	100c	94c	24a
5 mg/kg	131a	131b	127b	126ab	114b	101c	103ab	21ab
10 mg/kg	134a	137a	136a	132a	123a	111a	109a	18b
25 mg/kg	132a	123c	122c	125b	115b	106b	101bc	23ab

[y] Values in columns with the same letter are not significantly different ($P > 0.05$).
[z] % decrease in loaf volume between day 0 and day 34 of refrigerated storage.

GOX allowed the loaves baked from refrigerated dough to maintain their volume until day 6 of storage. In contrast, loaves prepared with 25 mg/kg of GOX began to show decrease in volume after only one day of storage.

Dough syruping was also evident in the dough prepared for the pup loaves. During storage, water was released from the dough, forming the syrup that coated the outside of the dough and making it sticky. The loss of the water from the dough as well as the loss of dough strength caused the baked loaves to have a dense crumb. The crust of the pup loaves became increasingly dark during the storage period because of the syrup forming on the outside of the dough.

Protein Quality. HP-SEC has been extensively used to analyze molecular weight distribution of wheat proteins (Bietz 1984; Ohm et al 2009). Gluten proteins are a heterogeneous class with a mixture of polymeric glutenin having molecular weight from 80,000 to several million and monomeric gliadins of molecular weights ranging from 30,000 to 80,000. Albumins and globulins are non-gluten proteins whose molecular weights were reported to be <25,000 (Veraverbeke and Delcour 2002). SDS buffer extractable and unextractable proteins were analyzed by HP-SEC. The analysis of wheat proteins with HP-SEC exhibited six main fractions (F1–F6) that corresponded to different protein classes. These were as follows: F1, high-molecular-weight polymeric protein (3.6–4.3 min retention time); F2, low-molecular-weight polymeric protein (4.3–5.87 min); F3, gliadin I (5.87–6.25 min); F4, gliadin II (6.25–6.9 min); F5, albumin and globulin (6.9–8.07 min); and F6, soluble protein (8.07–8.45 min) (Ohm et al 2009).

Figure 5 shows the average HP-SEC profiles of total extractable proteins from refrigerated dough with GOX during 16 days of the 34 day storage period. During storage the absorbance areas for the polymeric proteins (F1 and F2) decreased, whereas the absorbance areas for albumin and globulin and other soluble proteins (F5 and F6) increased for all GOX treatments. These results showed that during refrigerated storage of the dough there was degradation of the proteins that was not completely prevented by the addition of GOX. These results were in agreement with changes in the rheological properties of dough. The results obtained in the present work appear to reflect protein–protein interaction, and they can be relevant to affect the rheology of dough, because changes were also determined in the protein upon storage. There were no significant differences in the absorbance areas among the levels of GOX treatment, so the results were averaged across treatments. The lack of differences among GOX treatments for protein quality in the refrigerated dough may be because of
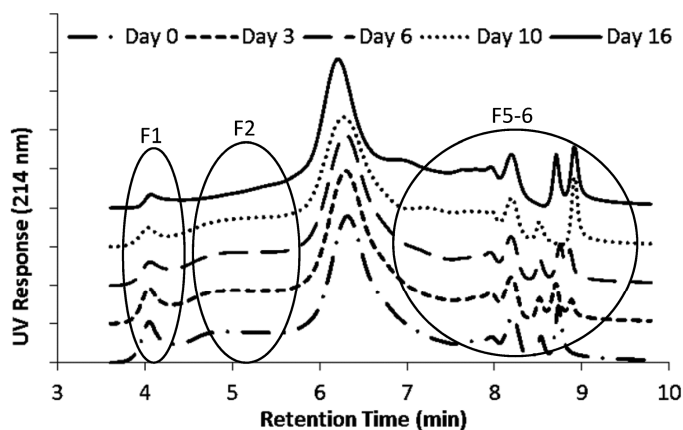
lack of substrate (Wikström and Eliasson 1998), because no additional glucose was added to the formula.

The correlation spectrum obtained over individual retention time intervals showed high enough resolution to identify protein fractions that had significant associations with quality characteristics. The absorbance area values of the main protein fractions (F1–F6 in Fig. 5) were also calculated. Retention time ranges of F1–F6 sections were determined based on their associations with quality characteristics found in previous research by Morel et al (2000) and Ohm et al (2006, 2009). Figure 6 shows the correlation between absorbance area of total proteins and DDS (Fig. 6A) and loaf volume (Fig. 6B) in refrigerated dough with GOX. Highly ($P < 0.01$) and very highly ($P < 0.001$) significant correlations were found between the changes in protein composition and the DDS as well as the loaf volume.

There were highly significant ($P < 0.01$) and very highly significant ($P < 0.001$) negative correlations between the DDS and absorbance area of the high-molecular-weight polymeric proteins and the absorbance area of the low-molecular-weight polymeric proteins, respectively. The absorbance area for the gliadins (F3 and F4) also had very highly significant ($P < 0.001$) negative correlation with DDS. The negative correlation indicated that during storage as more of the protein was hydrolyzed there was an increase in DDS. This result showed that there was degradation of the gluten-forming proteins in the refrigerated dough during storage. Therefore, part of the water released in the syrup during stor-
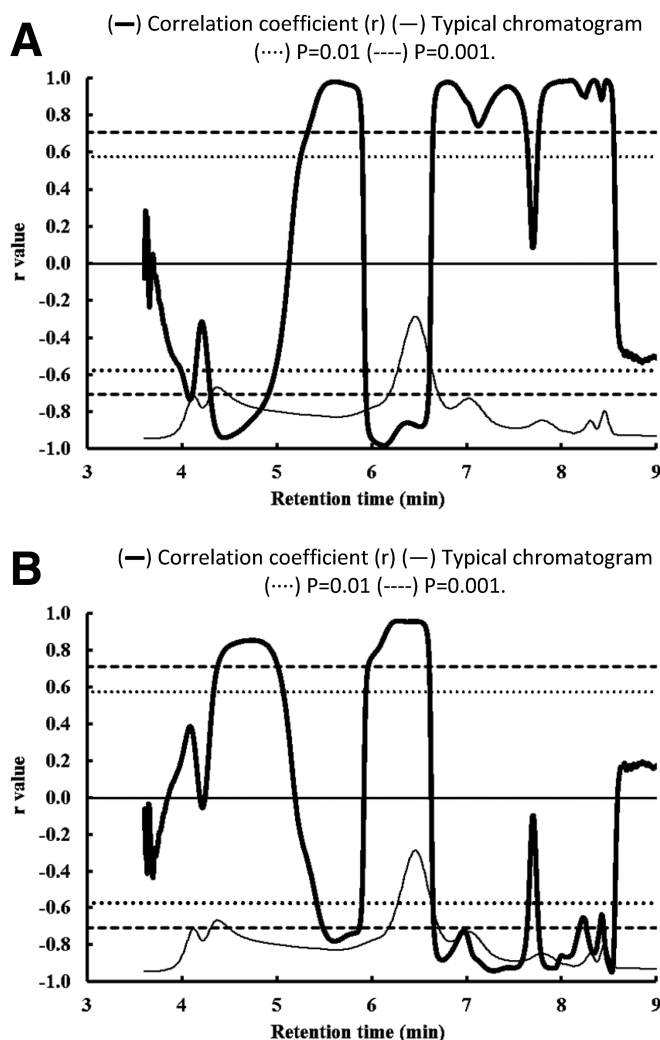

Fig. 6. Correlation between absorbance area of total proteins and degree of dough syruping (**A**) and loaf volume (**B**) in refrigerated dough with glucose oxidase.

Fig. 5. Average high-performance size-exclusion chromatography (HP-SEC) profiles of SDS-extractable proteins from refrigerated dough with glucose oxidase. HP-SEC profiles are average of all glucose oxidase treatment levels. F = fraction; F1 = high-molecular-weight polymeric protein (3.6–4.3 min retention time); F2 = low-molecular-weight polymeric protein (4.3–5.87 min); F5 = albumin and globulin (6.9–8.07 min); and F6 = soluble protein (8.07–8.45 min).

age resulted from loss of water-holding capacity of the gluten proteins. Conversely, the DDS had very highly significant ($P <$ 0.001) positive correlation with the globulins and albumins and other soluble proteins (F5 and F6). The absorbance areas of these fractions increased because of the hydrolysis of the gluten-forming proteins (F1 and F2).

The hydrolysis of the proteins in the dough during storage also had an effect on the end-product quality of the refrigerated dough. The absorbance areas of the protein in the refrigerated dough also had very highly significant ($P <$ 0.001) correlations with the loaf volume (Fig. 6B). The absorbance areas for low-molecular-weight polymeric proteins (F2) and gliadins (F3 and F4) had very highly significant ($P <$ 0.001) positive correlation with loaf volume. On the other hand, loaf volume had very highly significant ($P <$ 0.001) negative correlation with the absorbance area for albumins and globulins and other soluble proteins (F5 and F6). The viscous and elastic nature of wheat proteins were largely influenced by the ratio of the specific protein fractions contained in the wheat. Changes in the amounts of the protein fractions will lead to variations in the viscoelasticity of the dough, which ultimately affects the end-product quality (i.e., loaf volume) (Payne 1987).

CONCLUSIONS

The addition of GOX resulted in reduction in DDS and helped to maintain the strength and rheological characteristics of refrigerated dough. However, formulation of refrigerated dough products with GOX must be done carefully. Addition of excessive GOX to the dough will result in higher DDS and so may not compensate for loss of dough strength. In this study, 10 mg/kg addition of GOX was found to be optimal. Substantial degradation of protein in the refrigerated dough was also observed. The composition of the proteins in the refrigerated dough was found to be related to DDS and loaf volume. The DDS was negatively correlated to the polymeric proteins and positively correlated to low-molecular-weight proteins. However, the loaf volume showed the opposite trend as that of the correlation between DDS and protein composition. This observation means that as the proteins are hydrolyzed during refrigerated storage the DDS will increase and the volume of the baked product will decrease. Overall, GOX can be used to improve the quality of refrigerated dough products.

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